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14. ABSTRACT
Two antileishmanial test systems (MLS and MLL) were used to evaluate compounds at one or more levels. These test systems used female BALB/c mice infected with Leishmania major. There were 11 MLS tests done and 242 compounds used. The following 19 compounds were active; BU55197, BU5504,BP20206, BQ90098, BQ90552, BS81946, BS83191, BS86085, BU30118, BQ90981, BQ92145, AQ52825, BS04930, BS80690, BS93553, BU26730, BU59640, BS84858 and BU68416. In the MLS test parasites were injected intradermally (ID) at the base of the tail. Drug treatment was started on day 3 post infection IP for 10 days. In addition, 13 MLL tests were done and 113 compounds used. Results indicated 4 actives: BG32694, BU59640, BU59640 and BU68452. In the MLL test, mice were infected ID at the base of the tail and treatment was started IP for 10 days when the lesions reached between 20-70 mm2. AmBiosome was used as the positive control for all test systems. The Oracle database was used for tabulation of data and statistically analyzing the results.

15. SUBJECT TERMS- Leishmania major, Cutaneous Leishmaniasis, antileishmanial drugs

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INTRODUCTION

Leishmaniasis is a disease that has several different types of clinical pathology ranging from visceral pathology to simple cutaneous lesions. Cutaneous Leishmaniasis It has been around for centuries and remains a serious problem in areas such as Iraq, Afghanistan, Iran, Saudi Arabia, Yemen, Peru and Brazil. The US Army has had over 3,500 troops infected with this protozoan parasite. Kabul is believed to have the greatest number of cutaneous leishmania cases in the world with an incidence of over 67,000 cases per year. It is also found in more than 70 countries in the world. In the South American country of Colombia a large numbers of cutaneous leishmania cases (over 40,000) were reported between 2005 and 2009. It was caused by *Leishmania braziliensis* and many dogs serve as reservoir hosts that the vector (a sand fly) bites and gets infected then bites a human and transmit the disease. There is no vaccine for this parasite in humans so treatment relies on chemotherapy. The treatment for this disease is complicated because of several reasons; long treatment schedules, toxic drugs with many serious side effects, they are becoming less effective and many are expensive. Pentavalent antimonials are one of the major drugs used today but toxicity is a problem, resistance has developed and it must be administered by trained medical personnel for a prolonged time period. Miltifosine (an Alkyl-lysopholipid) is an oral preparation that has low efficacy and resistance has developed to it. Amphotericin B intravenous formulations are used in many areas of the world but they are very expensive and there are many side effects. Several other parental drugs such as pentamidine and oral triazoles like fluconazole and itraconazole have been used but their efficacy is still not very good. Paromomycin has been shown to be effective in some cases when administered topically in combination with Gentamicin. The majority of cases of cutaneous leishmaniasis from the Middle East are cause by Leishmania *major* and new therapies are desperately needed. We are using this parasite in a rodent model to find new effective chemotherapeutic agents.

BODY

There were 2 mouse models of cutaneous antileishmanial used in female BALB/c mice infected with *Leishmania major*. The first test is the Mouse Leishmania Suppressive (MLS) test system. The second test system tested compounds found active in the MLS test is called the Miami Leishmania Lesion (MLL) test system. Metacyclic parasites (1 X 106) obtained from cultures of donor mice infected footpads were used as an inoculum for each test. In the MLS test model and the MLL model the mice were injected with the parasites intradermally (ID) in the shaved area of the back about 1 inch forward from the tail. Test compounds were administered intraperitoneally (IP) once a day for 10 days starting 3 days post infection for the MLS tests and once a day for 10 days IP to mice in the MLL test once the developing lesion sizes were between 20-70 mm2. The lesions were measured on days 14, 21and 28 days after infection for the MLS test. For the MLL test the lesions were measures at the start of treatment then on days 7, 14, 21 and 28. The female BALB/c mice were weighed on the day of infection and daily during treatment then once a week for the duration of the MLS test. For the MLL test the mice were weighed on the day of infection then weekly until administration of test compounds. They were then weighed daily during treatment and then weekly until the end of the test. All of the data was entered into an Oracle database and at the conclusion of each test and an analysis of the data was made and a % suppression of the lesion sizes in both the MLS and MLL tests. In both the MLS and MLL tests the data obtained from the treated mice where compared with the infected non-treated control mice. The positive control was AmBisome at 12.5 mg/kg/day for 10 days in the MLS test and 37.5 mg/kg/day for 10 days in the MLL test. In the MLS and MLL tests compounds were considered active if the lesion size was suppressed by at least 50%.

For the MLS 242 compounds were tested and 19 were found to be active. There were 133 compounds tested in the MLL test system with only 13 exhibiting activity.

KEY RESEARCH ACCOMPLISHMENT

- Performed 11 MLS tests evaluating 242 compounds and found 19 actives.
- Performed 13 MLL tests evaluating 133 compounds and found 4 active.

- The new Oracle data base developed by WRAIR for data obtained in the Leishmanial MLS and MLL Test Systems was modified to enable changes in the protocol to be incorporated and it worked well for the following activities measured; mortality, mouse body weights, survival times, clinical pathology, toxicity, statistics including the % suppression of lesion size which was used to determine if a compound was active (need at least a 50% suppressive figure to be considered an active compound).

REPORTABLE OUTCOMES

The Oracle database developed by WRAIR for entering and analyzing data obtained from the Antileishmanial MLS and MLL Test Systems and was further validated. This database allows one to enter data for the MLS and MLL test systems as it is obtained directly into the database and analyzes it. Such data includes mouse body weights, drugs and their dosages, clinical observations of the mice, % suppression of lesion size, toxicity and mortality values. A similar database is being developed for the data obtained from the drug combination tests in the MLS and MLL Test Systems and the Miami Leishmania Hamster test system using *L. panamensis*.

There were 11 MLS tests where 242 compounds were tested with 19 active. In the MLL test system there were 13 tests with 133 compounds and found 4 active.

CONCLUSIONS

Two antileishmanial drug test systems (MLS and MLL) were validated in mice infected with *Leishmania major*.

Nineteen active compounds were found out of 242 in the MLS system. In the MLL system there were 4 active out of 133 compounds.

The Oracle Data Base System has been updated and validated to enter and analyze data for the MLS and MLL Test Systems. This data base allows all of the data in this system to be computerized and made available to Army researchers at Walter Reed on a regular basis.

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APPENDICES

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Arba Ager[1], Bill Ellis[2], Juan Mendez[2], Richard Boodoo[1], Jenbon Lui[1], Max Grogl[2] **CUTANEOUS LEISHMANIA MODEL IN BALB/C MICE WITH LEISHMANIA MAJOR TO DETECT NEW ANTILEISHMANIAL COMPOUNDS. Am. Soc. Trop. Med. & Hyg. New Orleans, LA, 2008**

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20. Other works <u>accepted</u> for publication:

None

PROFESSIONAL

21. Editorial Responsibilities: Acta Tropica

22. Professional and Honorary Organizations (member; officer; date):

American Society of Microbiology
American Society of Parasitology
American Society of Tropical Medicine and Hygiene

23. Honors and Awards:

World Health Organizations Special Training Fellowship, April, 1978. University of Liverpool, England, Department of Tropical Medicine and Parasitology.

National Institute of Health Malariology Traineeship Fellow, University of Georgia, 1969-1972

Sociedad Ecuatoriana de Medicina Tropical *y* Parasitologia. (Honorary Member). 1988.

24. Other Professional activities (e.g., papers presented; performances; conference proceedings; seminar or conference panel member; catalogue work; etc.):

Conferences

First Rodent Malaria Genomics Meeting, Atlanta, Ga. Presented a paper entitled: The role of rodent malaria in antimalarial drug screening. 2001.

First American and Caribbean Workshop on Emerging and Re-emerging Diseases.
Tegucigalpa, Honduras. Presented a paper entitled: Malaria: Drug resistance and control in Central America. 1997.

Invited outside lectureships

Lectureships in Medical Parasitology, University of the West Indies, School of Medicine, Kingston, Jamaica, 1978, 1979, 1980, 1981.

Lectureship in Medical Parasitology, American University of the Caribbean, School of Medicine, Montserrat, 1982.

Consultantships

Consultant in Medical Parasitology to Universidad Cayetano Heredia, Lima, Peru.

25. TEACHING

Teach Medical Parasitology to MD students as part of MIC 501.

Teach Medical Parasitology to MD/MPH students

Teach Medical Parasitology MIC 322.

Teach Special Project Parasitology MIC 452 (Laboratory Course).

Teach Medical Parasitology part of MIC 301.

26. Thesis and Dissertation Advising/Post-doctoral student supervision (chairman or committee member; topic; student name; date):

David Lowery, Ph.D. June 1985.
Mary Kalscheuer, M.S. Biology Department, 1986.
Jorge Bonilla, Ph.D. Dept of Pathobiology, University of Florida 2004.
Tereza Magalhaes, Ph.D. Dept of Epidemiology 2005.

Adriana Troyo, Ph.D. Dept. of Epidemiology 2007.

Appendix *Leishmania*

TABLE 1 MLS RESULTS

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
960	1	BQ97891	10	-52
	2	BS80118	160	-77
	3	BS82658	80	-151
	4	BS83397	20	-61
	5	BS83637	80	-85
	6	BS83646	80	-20
	7	BS84027	10	-151
	8	BS85293	40	-3
	9	BS85355	40	-192
	10	BU24067	20	-13
	11	BU30654	160	-18
	12	BU55197	160	56
	13	BU55204	160	100
	14	BS82354	80	-76
	15	BS79660	15	-8
	16	AF84868	80	-6
	17	BD36704	160	-88
	18	BM18573	160	-2
	19	BN34143	40	1
	20	BN43106	20	-87
	21	BN62067	160	38
	22	BN68532	5	-21
	23	BS94407	12.5	100

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
961	1	BN79704	20	Toxic
	2	BP16828	160	26
	3	BP20206	80	59
	4	BP21187	160	-29
	5	BQ35168	20	-48
	6	BQ35346	160	36
	7	BQ35382	160	33
	8	BQ35435	160	-29
	9	BQ90098	40	67
	10	BQ90552	10	51
	11	BQ90605	80	22
	12	BQ91942	80	-13
	13	BQ93197	40	-14
	14	BQ94836	10	-44
	15	BQ95235	40	14
	16	BQ95397	5	-17
	17	BQ95548	160	-54
	18	BQ95860	20	-66
	19	BQ96303	40	-59
	20	BR36919	80	-2
	21	BS81946	160	100
	22	BS82265	160	-14
	23	BS94407	12.5	100

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
969	1	BS83191	160	51
	2	BS83413	160	37
	3	BS84296	5	25
	4	BS84732	10	-2
	5	BS85051	10	20
	6	BS85999	80	25
	7	BS86085	80	73
	8	BS86245	40	-4
	9	BS89442	80	-6
	10	BS89951	10	20
	11	BS93384	40	12
	12	BS93768	160	-65
	13	BS96474	160	-37
	14	BU25304	10	-11
	15	BU25939	10	-30
	16	BU30118	40	53
	17	BQ90767	80	34
	18	BQ90794	5	-34
	19	BQ90810	20	9
	20	BQ90838	20	18
	21	BQ90981	160	52
	22	BQ91086	10	-27
	23	BS94407	12.5	97

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
970	1	BQ91540	160	6
	2	BQ91602	80	23
	3	BQ92029	160	-63
	4	BQ92083	160	-13
	5	BQ92145	160	54
	6	BQ92494	160	7
	7	BQ92761	10	14
	8	BQ93142	20	-20
	9	BQ93160	80	-47
	10	BQ93188	160	-23
	11	BQ93375	160	25
	12	BQ93400	160	-20
	13	BQ93446	10	7
	14	BQ93482	80	-36
	15	BQ93642	160	-30
	16	BQ93688	40	-45
	17	BQ93768	40	-30
	18	BQ94345	5	23
	19	BQ94381	40	-99
	20	BQ94416	40	-58
	21	BQ94443	20	-27
	22	BQ94452	20	12
	23	BS94407	12.5	100

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
971	1	BQ94461	80	-86
	2	BQ94470	10	-31
	3	BQ94505	80	-12
	4	BS83495	80	-92
	5	BS83575	10	-73
	6	BU25957	20	40
	7	BU30047	20	-51
	8	BQ91219	40	-29
	9	BQ91264	20	-5
	10	BQ91291	40	-66
	11	BQ91308	160	-65
	12	BQ91317	160	43
	13	BQ91399	80	-65
	14	BQ91433	20	-101
	15	BQ91451	80	-19
	16	BQ91479	4	-134
	17	BQ92798	20	Toxic
	18	BQ93035	20	-30
	19	BQ93295	10	21
	20	BQ93339	40	-33
	21	BQ93491	160	-103
	22	AG03635	10	-36
	23	BS94407	12.5	99

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
972	1	AJ64549	40	-39
	2	AR12559	80	4
	3	AW46149	10	-1
	4	BJ01377	16	1
	5	BM39769	20	-66
	6	BQ94498	40	33
	7	BQ94514	16	31
	8	BQ94569	20	-9
	9	BQ94578	10	11
	10	BQ94587	20	-67
	11	BQ94783	10	-71
	12	BQ94809	10	12
	13	BQ94818	20	-15
	14	BQ94854	2.5	-50
	15	BQ94872	20	-117
	16	BQ94916	5	-44
	17	BQ94934	160	-91
	18	BQ95137	10	-110
	19	BQ95164	10	-67
	20	BQ95379	10	-66
	21	BQ95404	20	-15
	22	BQ95511	40	-76
	23	BS94407	12.5	100

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
973	1	BQ95628	40	-17
	2	BQ95691	160	8
	3	BQ95717	20	-20
	4	BQ95799	10	15
	5	BQ96107	40	-1
	6	BQ96125	160	-3
	7	BQ96223	160	-14
	8	BQ96232	40	-5
	9	BS05259	10	-8
	10	BS80529	160	-14
	11	BS80583	40	Toxic
	12	BS80592	5	-11
	13	BS83360	160	11
	14	BS83904	80	-51
	15	BS83959	40	-52
	16	BS85257	10	-10
	17	BU24594	160	0
	18	BU29357	5	-88
	19	BU30109	80	-17
	20	BU30243	40	-65
	21	BQ92547	2.5	-4
	22	BQ94701	80	27
	23	BS94407	12.5	100

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
985	1	BU24281	40	18
	2	BS93740	160	29
	3	BS94069	40	17
	4	BS96670	160	-5
	5	AR49261	40	43
	6	AK55401	5	28
	7	AK78459	40	Toxic
	8	AQ52825	20	88
	9	BQ91424	20	27
	10	BQ97220	20	23
	11	BQ99215	2.5	23
	12	BS04930	20	50
	13	BS05268	5	16
	14	BS57459	160	47
	15	BS79464	160	20
	16	BS79624	80	-51
	17	BS80690	80	51
	18	BS82470	80	18
	19	BS83299	5	Toxic
	20	BS85284	160	-43
	21	BS88132	2.5	-57
	22	BS92449	20	-13
	23	BS94407	12.5	100

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
989	1	BS93553	5	100
	2	BS94265	40	41
	3	BS96189	20	-10
	4	BU24790	10	5
	5	BU26730	160	78
	6	BU59640	40	100
	7	BU60063	20	-75
	8	BH13827	20	47
	9	BM36240	80	-4
	10	BQ96296	160	-2
	11	BQ96483	160	-11
	12	BS82005	80	-23
	13	BS82032	160	38
	14	BS82210	80	7
	15	BS83440	80	-16
	16	BS83548	160	-8
	17	BS84652	80	-6
	18	BS85131	160	-18
	19	BS85168	20	32
	20	BU22223	40	27
	21	BU23177	160	-25
	22	BU23239	20	-46
	23	BS94407	12.5	100

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
990	1	BU24325	80	15
	2	BQ97248	80	-81
	3	BS82489	80	-7
	4	BS82505	40	6
	5	BS84858	60	54
	6	BS85408	40	-1
	7	BS85757	160	-21
	8	BU28421	20	9
	9	BS82336	80	3
	10	BS84849	20	-74
	11	BS86192	40	0
	12	BS88016	10	-70
	13	BU23720	40	37
	14	BQ97613	40	2
	15	BQ97640	20	-45
	16	BQ97659	40	-35
	17	BS82550	40	Toxic
	18	BS82612	40	38
	19	BS83271	80	-34
	20	BS83342	40	-51
	21	BS83708	80	-18
	22	BS83806	80	-32
	23	BS94407	12.5	100

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
991	1	BS83959	20	-9
	2	BS84741	160	28
	3	BS85202	160	-2
	4	BS86254	20	9
	5	BS92501	60	Toxic
	6	BS96483	80	-270
	7	BU22876	20	15
	8	BU24085	20	-55
	9	BU24101	20	-21
	10	BU26114	40	-32
	11	BQ99242	2.5	-42
	12	BQ99279	20	11
	13	BR29496	10	-29
	14	BR29521	10	-27
	15	BR29567	10	25
	16	BS82621	80	-84
	17	BU68407	40	-7
	18	BU68416	20	99
	19	BU68425	160	6
	20	BU68434	160	8
	21	BU68443	5	-64
	22	BU68452	80	3
	23	BS94407	12.5	100

TABLE 2 MLS ACTIVES

TEST		COMPOUND	MG/KG/DAY	% SUPPRESSION
960		BU 55197	160	56
		BU 55204	160	100
960		BP 20206	80	59
		BQ 90098	40	67
		BQ 90552	10	51
		BS 81946	160	100
969		BS 83191	160	51
		BS 86085	80	73
		BU 30118	40	53
		BQ 90981	160	52
970		BQ 92145	160	54
985		AQ 52825	20	88
		BS 04930	20	50
		BS 80690	80	51
989		BS 93553	5	100
		BU 26730	160	78
		BU 59640	40	100
990		BS 84858	60	54
		BU 68416	20	99

TABLE 3 MLL TESTS

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
965	1	AB11829	160	-19
	2	AB65390	160	-5
	3	AR18631	40	3
	4	AR18631	20	3
	5	AR18631	10	3
	6	BQ91102	20	4
	7	BQ92001	40	-2
	8	BQ92001	20	-2
	9	BQ92001	10	-2
	10	BQ92314	40	-7
	11	BQ95833	40	19
	12	BQ95833	20	19
	13	BQ95833	10	19
	14	BS84983	160	-9
	15	BS94407	37.5	100

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
966	1	BS84821	40	16
	2	BS84821	20	16
	3	BS84821	10	16
	4	BS82247	80	1
	5	AC97879	80	6
	6	AT29027	80	7
	7	BG21744	20	-17
	8	BG21744	40	2
	9	BQ93179	40	-3
	10	BQ93795	40	-9
	11	BQ93795	40	-9
	12	BQ94532	80	-3
	13	BQ97506	160	Toxic
	14	BS79786	40	-21
	15	BS80029	20	15
	16	BU25331	40	17
	17	BS94407	37.5	100

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
967	1	BG32694	80	1
	2	BH30346	10	6
	3	BL23622	160	10
	4	BM13827	80	-2
	5	BN08009	80	7
	6	BQ35177	10	20
	7	BQ93624	80	-12
	8	BQ95762	40	-1
	9	BQ97186	10	4
	10	BS83244	10	-13
	11	BS85444	10	34
	12	BS88025	80	5
	13	BU25180	40	26
	14	BU27997	40	Toxic
	15	BS94407	37.5	100

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
976	1	BU30574	40	-8
	2	BG32694	80	-27
	3	BL23622	160	-3
	4	BM13827	80	20
	5	BN08009	80	10
	6	BQ93624	80	-17
	7	BQ95762	40	-11
	8	BS88025	80	-16
	9	BU25180	40	15
	10	BU27997	40	4
	11	BU30574	40	-8
	12	BS94407	37.5	99

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
978	1	BQ35177	5	-2
	2	BS85444	5	Toxic
	3	BU27997	20	-5
	4	BS82247	40	-34
	5	AC97879	40	-33
	6	BQ97506	80	Toxic
	7	BQ97506	40	Toxic
	8	BS79786	20	-28
	9	BL23622	80	5
	10	BL23622	40	5
	11	BS88025	40	-26
	13	BS88025	20	-9
	14	BN08009	40	5
	15	BN08009	20	5
	16	BS94407	37.5	96

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
981	1	BG32694	40	50
	2	BG32694	25	50
	3	BG32694	15	50
	4	BM13827	40	37
	5	BM13827	20	37
	6	BM13827	10	37
	7	BM13827	5	37
	8	BU25180	20	33
	9	BU25180	10	33
	10	BU25180	5	33
	11	BS94407	37.5	100

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
983	1	BU59640	10	27
	2	BU59640	10	64
	3	BU59640	20	27
	4	BU59640	20	64
	5	BU59640	40	27
	6	BU59640	40	64
	7	BS94407	37.5	76

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
984	1	BU60072	20	0
	2	BU60072	40	11
	3	BU60081	0	2
	4	BU60081	0	-20
	5	BS94407	37.5	96

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
986	1	BG32694	40	22
	2	BG32694	25	22
	3	BG32694	15	22
	4	BM13827	40	22
	5	BM13827	20	22
	6	BM13827	10	22
	7	BM13827	5	22
	8	BU25180	20	28
	9	BU25180	10	28
	10	BU25180	5	28
	11	BS94407	37.5	99

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
987	1	BS83191	160	-30
	2	BS83413	160	-58
	3	BS84296	5	-50
	4	BS86085	40	-35
	5	BS85999	80	-28
	6	BS89951	10	-59
	7	BU30118	40	-63
	8	BQ90981	160	-32
	9	BS94407	37.5	85

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
988	1	BS94407	37.5	82
	2	BU59640	40	67
	3	NONE	0	
	4	BQ95762	20	16
	5	BQ95762	10	16
	6	BQ95762	5	16
	7	BQ93624	40	23
	8	BQ93624	20	23
	9	BQ93624	10	23
	10	BN62067	80	8
	11	BU55197	80	7
	12	BU55204	80	6

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
993	1	BU68407	40	-31
	2	BU68416	20	14
	3	BU68425	160	-10
	4	BU68434	160	-14
	5	BU68443	5	22
	6	BS94407	37.5	100

TEST	GROUP	COMPOUND	MKD	% SUPPRESSION
996	1	BU68452	80	64
	2	BU59640	40	-1
	3	BS93553	5	-4
	4	BS81508	160	-4
	5	BQ93786	40	-22
	6	BU26730	80	-28
	7	BQ93759	80	-29
	8	ZP74275	10	-17
	9	BU30190	160	-17
	10	ZW61835	160	-22
	11	BS94407	37.5	100

TABLE 4 MLL ACTIVES

TEST		COMPOUND	MKD	% SUPPRESSION
981		BG 32694	40	50
983		BU 59640	40	64
988		BU 59640	40	67
996		BU 68452	80	80